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Structural Diversity of Ascidian Crystalline Celluloses*¹

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Tunic is an extracellular matrix covering the monolayered epidermis of tunicates (Ascidian). An interesting feature of the tunic is the composite structure of fibrous cellulose and protein matrix. This animal cellulose has been known to be similar to the higher plant cellulose but with extremely highly crystalline¹⁾. At present, native cellulose is found to be a composite of two distinct crystalline modifications namely cellulose I_α and I_β²⁾, whose corresponding crystal structures are one-chain triclinic unit cell and two-chain monoclinic unit cell, respectively³⁾. In this scheme all the native celluloses are roughly categorized into two groups; I_α rich algal-bacterial type and I_β dominant cotton-ramie type. To date, tunicate celluloses from two origins^{4,5)} are investigated and found to be unique in that they consist of pure I_β because most of the cotton-ramie type celluloses are known to contain small portion of I_α as well. In addition, the other crystallographic features such as dimension, orientation, and crystallinity, differs in relation to the cellulose origins. Therefore efforts to correlate such variation to the phylogeny of life has been underway particularly in the algal and plant celluloses. In this sense little is known about the tunicate celluloses.

Given this background, we have performed systematic investigation of tunicate celluloses in relation to its phylogeny. We analysed 40 species covering a wide range of the phylogenetic tree of Ascidian (including one from Desmomyatria, more primitive than Ascidian) by means of X-ray diffraction, electron microscopy, FT-IR and CP/MAS ¹³C NMR spectroscopy. Firstly, regarding the crystalline allomorphism, all the species investigated were found to be monoclinic I_β (cotton-ramie) type by the analysis of d-spacing data as well as the peak analyses of FT-IR and ¹³C NMR spectroscopy. Secondly, in some species 0.6 nm lattice planes were preferentially oriented with respect to the tunic surface.

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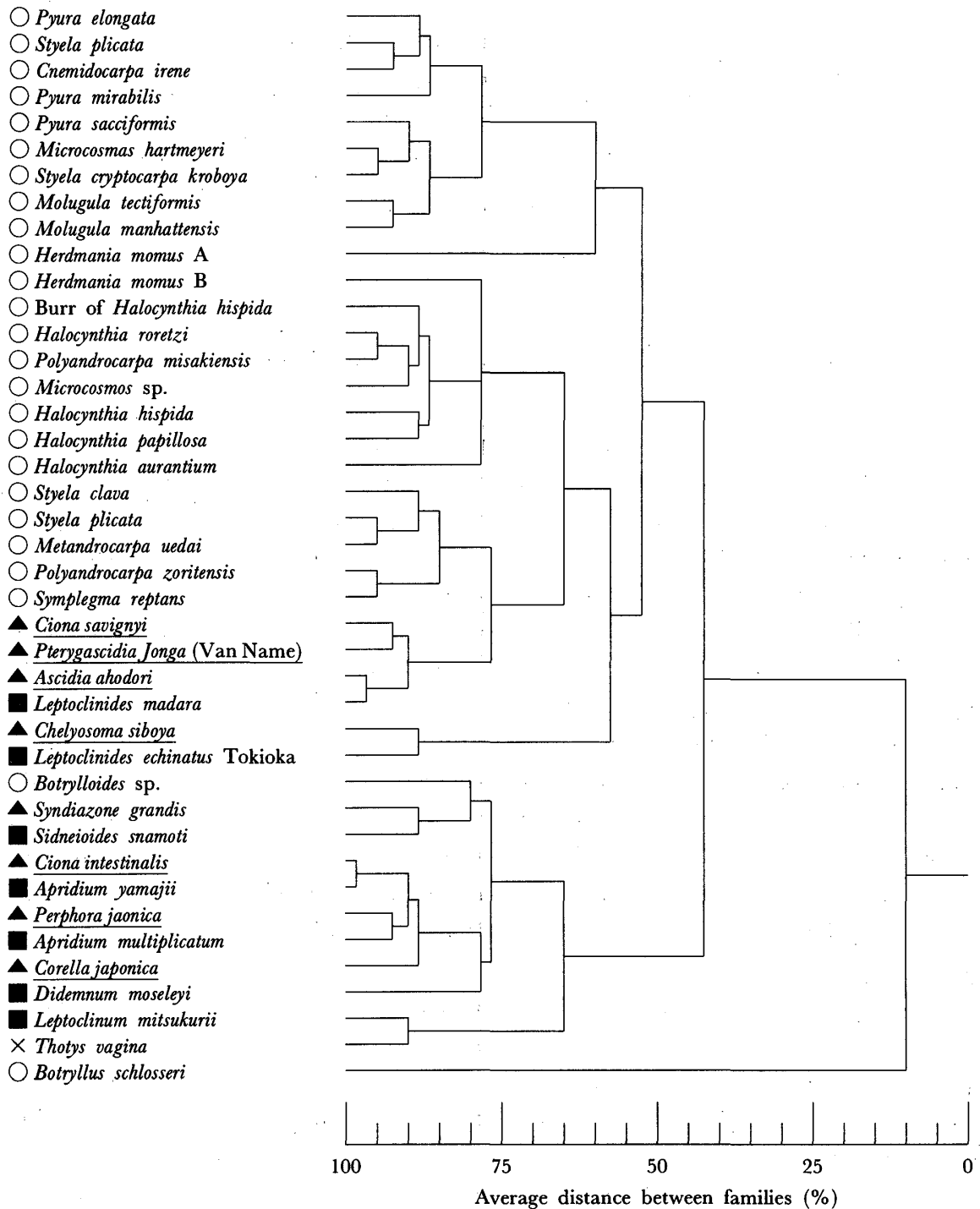


Fig. 1 A tree diagram of observed Ascidians on the basis of structural data of cellulose.
 Note: ○; Stolidobranchiata, ▲; Phebobranchiata, ■; Aplousobranchiata, ×;
 Desmomyatria. From the top to *Leptoclinides echinatus*: solitary Ascidian. The rest expect for
Thetys vagina: colonial ascidian: Thetys is a solitary plancton-type tunicate.

Finally, multivariate analysis, on the basis of numerical data set of microfibril width, relative orientation factor, and relative crystallinity index were conducted to measure the similarity or dissimilarity among the observed samples, and the results of cluster analysis was presented in Fig. 1. Interestingly, one of the three major groups (○; Stolidobranchiata) in Ascidian was grouped in the upper part of the tree, whereas the other two (▲; Phebo-branchiata, ■; Aplousobranchiata) were not well clusterized. However, taxonomically interesting data were generated:¹⁾ the variation in a give species was well classified (e.g. *Herdmania* and *Cionia*)²⁾. The solitary Ascidian were separated from the colonial Ascidian.

The ambitious aim of this study is to anticipate the evolutionary pathway of cellulose biogenesis to tunicates in terms of the cellulose structure. It is however the cellulose I_{β} with 0.6 nm-lattice-plane-oriented found in Ascidian is a unique, which has never been found in other cellulose synthesizing organisms.

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